

AMENDMENTS TO THE CLAIMS

- TECH CENTER BOOKSON (Currently amended) A method of producing rice plants from rice microspores comprising:
- selecting <u>rice</u> plant material including <u>rice</u> microspores at a developmental (a) stage amenable to androgenic induction;
- subjecting said microspores to temperature stress to obtain stressed (b) microspores;
- contacting said microspores with an amount of a sporophytic development (c) inducer effective to induce sporophytic development, said contacting step occurring before, during, after, or overlapping with any portion of said temperature stress step;
 - isolating said stressed and contacted microspores; [[and]] (d)
- (e) coculturing said isolated microspores with either ovary-conditioned medium or at least one live plant ovary; and
 - producing rice plants from the cocultured microspores.
- 2. (Original) The method of Claim 1 wherein the microspores within the selected plant material are in the mid uninucleate to early binucleate stage of development.
- 3. (Original) The method of Claim 1 wherein the microspores are subjected to temperature stress by incubating the selected plant material including said microspores at a temperature of from about 4°C to about 43°C.
- (Original) The method of Claim 3 wherein the selected plant material including 4. the microspores is incubated at a temperature of about 33°C.
- 5. (Original) The method of Claim 3 wherein the microspores are subjected to temperature stress for a period of from about half an hour to about 72 hours.

- 6. (Original) The method of Claim 1 further comprising the step of subjecting the microspores to nutrient stress by contacting the selected plant material including said microspores with an aqueous medium comprising an amount of at least one nutrient that is less than the amount of that nutrient necessary for the optimal growth and development of the microspores in the aqueous medium, said nutrient stress step occurring before, during, after, or overlapping with any portion of said temperature stress step.
- 7. (Currently amended) The method of Claim 6 wherein said aqueous medium comprises an at least 20% a dilution of NPB 98 that is less than or equal to 80% of its undiluted concentration.
- 8. (Original) The method of Claim 1 wherein said sporophytic development inducer is selected from the group consisting of 2-aminonicotinic acid; 2-chloronicotinic acid; 2-chloroethyl-phosphonic acid; 2-hydroxynicotinic 6-chloronicotinic acid; hydroxynicotinic acid; 3-hydroxypicolinic acid; Benzotriazole; 2-hydroxyproline; 2,2'-dipyridil; 2,4-pyridine dicarboxylic acid monohydrate; 2-hydroxypyridine; 2,3-dihydroxypyridine; 2,4dihydroxypyrimidine-5-carboxylic acid; 2,4-dihydroxypyrimidine-5-carboxylic acid hydrate; 2-hydroxypirimidine hydrate; 2,4,5-trihydroxypyrimidine; 2,4,6-trichloropyrimidine; 2-hydroxy-4-methyl pyrimidine hydrochloride; 4-hydroxypyrazolo-3,4,d-pyrimidine; quinaldic acid; violuric acid monohydrate; thymine; xanthine; salicylic acid; sodium salicylate; salicyl aldehyde; salicyl hydrazide; 3-chlorosalicylic acid; fusaric acid; picolinic acid; butanediene monoxime; di-2-pyridyl ketone; salicin; 2,2'-dipyridil amine; 2,3,5-triiodobenzoic; 2-hydroxy pyridine-Noxide; 2-hydroxy-3-nitropyridine; benzotriazole carboxylic acid; salicyl aldoxime; glycine; D Lhistidine; penicillamine; 4-chlorosalicylic acid; 6-aminonicotinic acid; 2,3,5,6-tetrachloride 4pyridine carboxylic acid; alpha benzoin oxime; 2,3-butadiene dioxime; isonicotinic hydrazide; cupferron; ethyl xanthic acid; 3-hydroxy benzyl alcohol; salicyl amide; salicyl anhydride; salicyl

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hydroxamic acid; methyl picolinic acid; 2-chloro pyridine; 2,6-pyridine carboxylic acid; 2,3-pyridine dicarboxylic acid; 2,5-pyridine dicarboxylic acid; pichloram; ammonium thiocyanate; amiben; diethyl dithiocarbamate; glyphosate; anthranilic acid; thiourea; 2,4-diclorophenoxyacetic acid; 4-chloro anisole; 2,3-dichloroanisole; 2-(2,4)-dichlorophenoxy propionic acid; 2-(4-chlorophenoxy)-2-methyl propionic acid; 2-(para-chloro phenoxy) isobutyric acid and α,β -dichlorobutyric acid.

- 9. (Original) The method of Claim 8 wherein said sporophytic development inducer is selected from the group consisting of 2-hydroxynicotinic acid, 2-chloroethyl-phosphonic acid, 2-chloronicotinic acid and 2-hydroxyproline.
- 10. (Original) The method of Claim 9 wherein said sporophytic development inducer is 2-hydroxynicotinic acid.
- 11. (Original) The method of Claim 9 wherein said sporophytic development inducer is 2-chloroethyl-phosphonic acid.
- 12. (Original) The method of Claim 1 wherein said sporophytic development inducer is present at a concentration of from about 0.001 mg/l to about 1000 mg/l.
- 13. (Original) The method of Claim 1 wherein said sporophytic development inducer is present at a concentration of from about 1 mg/l to about 500 mg/l.
- 14. (Original) The method of Claim 1 further comprising the step of contacting said microspores with an effective amount of an auxin, said step of contacting the microspores with an effective amount of an auxin occurring before, during, after, or overlapping with any portion of said temperature stress step.
- 15. (Original) The method of Claim 14 wherein said auxin is 2,4-dichlorophenoxyacetic acid.

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- 16. (Original) The method of Claim 14 wherein said auxin is utilized at a concentration of from about 0.1 mg/l to about 25 mg/l.
- 17. (Original) The method of Claim 16 wherein said auxin is utilized at a concentration of from about 0.5 mg/l to about 4.0 mg/l.
- 18. (Original) The method of Claim 1 further comprising the step of contacting said microspores with an effective amount of a cytokinin, said step of contacting the microspores with an effective amount of a cytokinin occurring before, during, after, or overlapping with any portion of said temperature stress step.
 - 19. (Original) The method of Claim 18 wherein said cytokinin is kinetin.
- 20. (Currently amended) The method of Claim 18 wherein said cytokinin is benzaminopurine consists essentially of benzylaminopurine.
- 21. (Original) The method of Claim 18 wherein said cytokinin is utilized at a concentration of from about 0.1 mg/l to about 10 mg/l.
- 22. (Original) The method of Claim 21 wherein said cytokinin is utilized at a concentration of from about 0.5 mg/l to about 2.0 mg/l.
- 23. (Original) The method of Claim 1 further comprising the step of contacting said microspores with an effective amount of a gibberellin, said step of contacting the microspores with an effective amount of a gibberellin occurring before, during, after, or overlapping with any portion of said temperature stress step.
- 24. (Original) The method of Claim 23 wherein said gibberellin is utilized at a concentration of from about 0.01 mg/l to about 20 mg/l.
- 25. (Original) The method of Claim 24 wherein said gibberellin is utilized at a concentration of from about 0.2 mg/l to about 4.0 mg/l.

26. (Original) The method of Claim 1 further comprising the step of contacting said microspores with an effective amount of a cell spindle inhibiting agent, said step of contacting the microspores with an effective amount of a cell spindle inhibiting agent occurring before, during, after, or overlapping with any portion of said temperature stress step.

27. (Original) The method of Claim 26 wherein said cell spindle inhibiting agent is utilized at a concentration of from about 1 μ M to about 200 μ M.

28. (Original) The method of Claim 26 wherein said cell spindle inhibiting agent is pronamide.

29. (Original) The method of Claim 1 wherein said stressed microspores are isolated by density centrifugation.

30. (Original) The method of Claim 29 wherein said density centrifugation utilizes a solution of mannitol layered over a higher density solution of maltose.

31. (Original) The method of Claim 1 wherein said coculturing step utilizes a liquid nutrient suspension medium selected from the group consisting of medium NPB98 and NPB-99.

32. (Original) The method of Claim 1 wherein said coculturing step utilizes at least one live ovary obtained from a plant variety selected from the group consisting of any wheat variety and barley variety Igri.

33. (Original) The method of Claim 1 wherein said coculturing step utilizes ovary-conditioned medium.

34. (Original) The method of Claim 1 further comprising the step of genetically transforming said microspores.

35. (Canceled)

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- 36. (Currently amended) A method of producing <u>rice</u> plants from <u>rice</u> microspores comprising:
- (a) selecting <u>rice</u> plant material including <u>rice</u> microspores at a developmental stage amenable to androgenic induction;
- (b) subjecting said microspores to temperature stress and nutrient stress to obtain stressed microspores;
- (c) contacting said microspores with an effective amount of an auxin, an effective amount of a cell spindle inhibiting agent and an effective amount of a sporophytic development inducer, said contacting step occurring before, during, after, or overlapping with any portion of said temperature and nutrient stress step;
 - (d) isolating said stressed and contacted microspores; [[and]]
- (e) coculturing said isolated microspores with either plant ovary conditioned medium or at least one live plant ovary; and
 - (f) producing rice plants from the cocultured microspores.